Clin Exp Immunol 1993; **94**:80–83

Ascaris reinfection of slum children: relation with the IgE response

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(Accepted for publication 5 July 1993)

SUMMARY

Total and *Ascaris*-specific serum IgE levels were measured in a group of 98 *Ascaris*-infected children from a slum area of Caracas, Venezuela, in whom the infections were eliminated by regular treatment for 22 months with the anthelmint Oxantel/Pyrantel ('Quantrel'). The children were re-evaluated at the end of the treatment programme, and then 8 months later, at which time reinfection was assessed. Total IgE levels at the beginning of the study were significantly higher in the children who became reinfected after treatment, compared with those who did not. The anthelmint treatment caused a significant decrease in the total IgE levels in most of the children, and after a period of 8 months without treatment these continued to decrease in the non-reinfected group, but increased again in the reinfected children. The reverse pattern was found for *Ascaris*-specific IgE antibody levels, and in fact an inverse correlation was found between total and anti-*Ascaris* IgE levels. Striking associations were found between reinfection and high pretreatment values of total IgE, but low levels of specific IgE antibody. These data support the concept that specific IgE antibody may participate in the protection against helminthic infection, and suggest that the polyclonal stimulation of IgE synthesis caused by these parasites may reduce the effectiveness of such responses. The results also indicate that different individuals have varying propensities to respond polyclonally to the helminths, and this influences their resistance to infection.

Keywords *Ascaris* IgE anthelmint treatment reinfection polyclonal stimulation helminthic infection

INTRODUCTION

The different helminthiasis are among the most prevalent of chronic infections of humans, and numerous studies have demonstrated that there is a process of constant reinfection in endemic areas [1,2].

One of the most interesting features of the immune response to these parasites is the production of IgE antibodies [3], and there exists considerable evidence that the IgE response may participate in the protection against helminthic infection [4–7]. For example, the allergic-type reactions mediated by the interaction between IgE and mast cells may be important accessory mechanisms in helminth expulsion [8], and more direct cellular cytotoxicity mechanisms have been described involving IgE and eosinophils [4].

Helminthic infection can also cause a non-specific polyclonal stimulation of IgE synthesis [3,9] that leads to highly elevated serum IgE levels in endemic populations [10–12]. This polyclonal stimulation may decrease the capacity to mount an effective specific IgE antibody response [9,13,14] and can cause mast cell saturation [13–16], thus suppressing the allergic response to environmental allergens, and possibly parasite antigens [13,14].

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In the present study we considered the possible role of the IgE response in the resistance to helminth infection in a group of urban slum children in Caracas, Venezuela.

SUBJECTS AND METHODS

Study group

A group of 98 children between 5 and 10 years of age (mean age $7{\cdot}5 \pm 3{\cdot}2$ years), initially infected with *Ascaris lumbricoides*, was studied in a slum area of Caracas, Venezuela (Barrio Los Erasos).

The children were evaluated before (T_0) and after (T_1) elimination of their infection by monthly treatment for 22 months with Oxantel/Pyrantel (Quantrel, Pfizer, New York, NY; 20 mg/kg). They were also evaluated 8 months after the end of the treatment programme (T_2), during which time they received no additional anthelmintic medication, and when approximately 50% of the group had become reinfected. Written permission of the legal representatives of the children was obtained, and the study was approved by the ethical committee of the Institute of Biomedicine. All the tests were performed under the supervision of the Children's Hospital of Caracas, through their medical dispensary in the slum area.

Table 1. Mean values of total and specific IgE and peripheral blood eosinophilia in the non-reinfected or reinfected children

	Reinfected ($n=52$)			Non-reinfected ($n=46$)		
	T_0	T_1	T_2*	T_0	T_1	T_2
Total IgE* (log U/ml)	3·75±0·34	3·56±0·32	3·67±0·30	2·98±0·38	2·75±0·40	2·59±0·52
Specific IgE† (log PRU/ml)	−0·52±0·04	−0·59±0·08	−0·57±0·09	−0·045±0·042	0·010±0·040	0·081±0·040
Eosinophilia‡ (%)	15·09±5·80	11·68±4·15	14·78±5·12	8·10±3·03	4·97±2·60	4·06±2·74

* T_0, Pre-treatment; T_1, end of 22 months of treatment; T_2, 8 months after end of treatment.
† Arithmetic mean±s.d. of log transformed values.
‡ Arithmetic mean±s.d.
PRU, Phadebas RAST units.

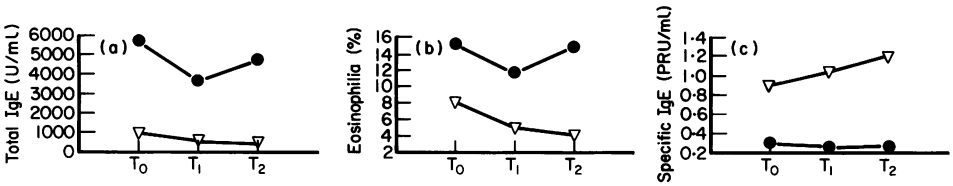

Fig. 1. Geometric mean of total serum IgE levels (a), mean eosinophilia (b) and geometric mean of specific anti-*Ascaris* IgE antibody (c) during the study period in children who either remained uninfected after treatment (▽) or became reinfected (●). T_0, Pre-treatment; T_1, end of 22 months of treatment; T_2, 8 months after end of treatment; PRU, Phadebas RAST units.

Faeces examination

Serial faeces samples were collected in preservative solution (40% tincture of merthiolate, 5% formalin, 1% glycerol) and examined for the presence of eggs or larvae of intestinal helminths. As either ascariasis or trichuriasis accounted for over 90% of the helminthic infections in the slum children, and the former was the most prevalent (70% and 35% respectively), only children who were infected with *Ascaris* were considered in this study. To assess the initial intensity of infection, the number of eggs per gram of stool was estimated in 61 children by the method of Stoll [17].

Serum IgE levels

The PRIST (Phadebas, Pharmacia, Sweden) technique for the measurement of total serum IgE levels was used, and the results expressed in U/ml.

A paper disk RAST technique [18] was used for the measurement of specific IgE against *Ascaris*, and while this test was developed in our laboratory, it was standardized against the commercial Phadebas test, and the results expressed as Phadebas RAST units (PRU/ml).

Blood eosinophilia

Samples of peripheral blood were smeared and air-dried on glass slides. Within a period of 1 month, differential counts of eosinophils were performed by counting a total of 200 leucocytes in the smears stained with Giemsa or Wright solutions.

Statistical analysis

The total and specific IgE levels were normalized by logarithmic transformation and the means±s.d. calculated. These data were back-transformed for the presentation of the data as the geometric means (g.m.). The mean IgE levels and the mean eosinophilias at the different times of evaluation were compared by Student's *t*-test, and the frequencies of helminthiasis among the groups by χ^2 analysis.

We also compared the proportion of reinfected children who had values of total and specific IgE above or below the group median.

Relationships with age were tested by both ANOVA and χ^2 analysis, and Spearman rank correlations were determined between specific and total IgE levels.

RESULTS

When the intestinal helminthic infections were eliminated in the group of 98 slum children by long-term anthelmint treatment, a reinfection rate of 52·6% for *Ascaris* was found upon re-evaluation at 8 months post-treatment.

For the purposes of the present analysis, at all time points we divided the children according to whether they were found to be reinfected by *Ascaris* 8 months after the end of anthelmint treatment, or remained uninfected. Possibly due to our intervention in the slum area over the past years, at the beginning of the study (T_0) the intensity of infection was found to be low, and not overdispersed, in both the children who subsequently became reinfected (620±40 *Ascaris* eggs/g of faeces) and those who did not (580±40 *Ascaris* eggs/g of faeces).

The log-transformed total and specific IgE levels were used for statistical analysis (Table 1), and the geometric means are presented in Fig. 1. The total serum IgE levels at T_0 were significantly ($P<0·005$) higher in the reinfected children, and by the end of the treatment (T_1) these had decreased significantly ($P<0·005$) in both groups (Fig. 1a). After a period of 8 months without anthelmint treatment (T_2), these levels continued to decrease significantly ($P<0·005$) in the non-reinfected group, whereas an increase occurred ($P<0·05$) in the reinfected children (Fig. 1a). The same general pattern was observed for blood eosinophilia (Table 1, Fig. 1b).

In contrast, when the serum levels of specific anti-*Ascaris* IgE antibody were measured, the reverse pattern was found

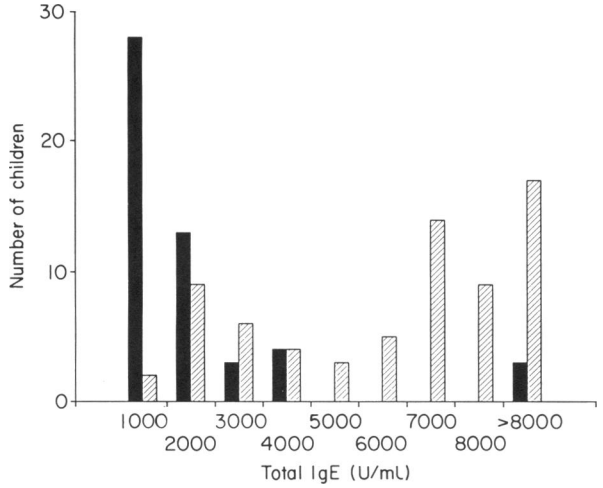

Fig. 2. Number of children non-reinfected (■) or reinfected (▨), according to the initial total serum IgE levels.

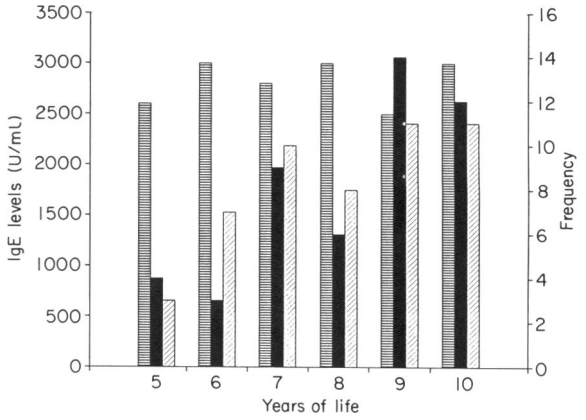

Fig. 3. The distribution of total IgE levels according to age (■), and the frequency of non-reinfection (■) or reinfection (▨) in the different age groups considered.

(Table 1, Fig. 1c). Thus, at the beginning of the study (T_0), these were significantly ($P < 0{\cdot}0001$) higher in the children who were not reinfected by T_2. By the end of the treatment period (T_1), these levels had increased significantly ($P < 0{\cdot}0001$) in the non-reinfected group, and after 8 months without treatment (T_2) they continued to increase in the absence of helminthic infection, but remained low in the reinfected children (Table 1, Fig. 1c). In fact, at all time points there were significant inverse correlations between total and specific IgE levels (for example, $r = -0{\cdot}69$, $P < 0{\cdot}001$, at T_0 for the whole group).

Figure 2 shows the frequency of reinfection in relation to the initial total IgE levels. In this respect it is important to note that 84% of the children who became reinfected had initial IgE levels that were above the median of the entire group (2150 U/ml), whereas in contrast, only 15% of the non-reinfected children had IgE levels that were above this value (χ^2 44·4, $P < 0{\cdot}001$). The reverse was the case for specific anti-*Ascaris* IgE antibody, because only 8% of the reinfected children had levels that were above the median (0·50 PRU/ml), compared with 81% of the non-reinfected group (χ^2 50·4, $P < 0{\cdot}001$).

It should be emphasized here that, at least for the age range evaluated, ANOVA and χ^2 analysis revealed no significant associations between age and either total IgE levels or reinfection (Fig. 3).

Finally, the children were grouped according to their household units, in order to determine whether familial clustering occurred in reinfection or elevated total IgE levels (Fig. 4). It can be seen that although there was wide variation in the total IgE levels and occurrence of reinfection within the individual family groups, the family members with the lowest initial total IgE levels were the least likely to become reinfected.

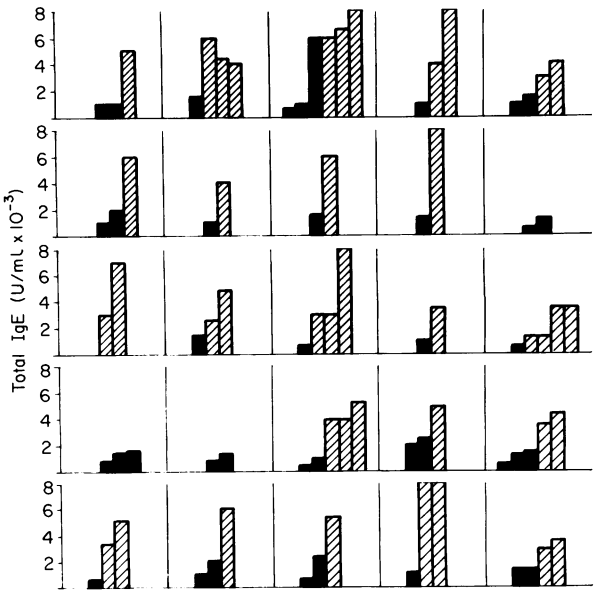

Fig. 4. Pretreatment total IgE levels in non-reinfected (■) or reinfected (▨) children belonging to different households in the study group. Each bar represents an individual child of the family group, and each panel represents a different family in the study group.

DISCUSSION

The production of large amounts of IgE is a characteristic feature of the immune response to helminthic infection [10,11]. This IgE is only in part specific for the parasite, and the remainder represents a non-specific polyclonal stimulation of this immunoglobulin [11,14,19,20]. It has been proposed that responses involving specific IgE participate in protective mechanisms against helminthic infection in man [4,6,7]. Concordant with this concept, we observed striking differences in the IgE response between slum children who were either reinfected or non-reinfected by *Ascaris* after anthelmint treatment. Thus, when a group of *Ascaris*-infected children were treated with Oxantel/Pyrantel on a monthly basis for a period of 22 months, then re-examined 8 months after treatment, the pretreatment total serum IgE levels were found to be significantly higher in the children who subsequently became reinfected. The same pattern was observed for blood eosinophilia. In contrast, the reinfection rate was lower in children with high initial levels of specific anti-*Ascaris* IgE antibody. In fact, a surprisingly clear separation between reinfection or non-reinfection was demonstrated depending on whether the starting IgE levels were below or above the group median values. In addition, in the group of

non-reinfected chldren, the total serum IgE levels decreased significantly after treatment, whereas in contrast the specific anti-*Ascaris* IgE antibody levels increased significantly and remained high during the course of the study. This was not the case for the reinfected children, who were unable to maintain an effective specific IgE response after anthelmint treatment, and whose total IgE levels showed a significant increase. These results support, therefore, a protective role of specific IgE antibody against *Ascaris*, and are in agreement with those reported by Hagan [7], where individuals with the highest levels of IgE antibody against either adult worms or egg antigens were less likely to be reinfected with *Schistosoma haematobium* after treatment than those with the lowest levels. Also, a negative correlation has been demonstrated between IgE against adult worm antigens of *S. mansoni* and reinfection [6].

Conversely, the polyclonal stimulation of IgE synthesis by helminths may interfere with the protective response. This concept is based on evidence that polyclonal IgE stimulation can suppress specific IgE responses to individual antigens [12–14], and can also inhibit the activity of mast cells via a saturation of their Fcε receptors [12–16]. In this respect, in the present study we found an inverse correlation between total and specific anti-*Ascaris* IgE, and have previously reported the existence of mast cell saturation in these slum children [13,21]. This suppression of allergic reactivity may reduce the pathological consequences of immune reactions against the parasite, or may represent a mechanism of parasite evasion, as has been proposed for IgG4 antibodies that block antibody-dependent killing of schistosomula by human eosinophils [5,6]. Thus, immunity to helminth infection may be a balance between the stimulation of immunological effector functions and the activation of blocking mechanisms. These results have potentially important implications for the control of helminthic infections in the community, in that treatment programmes that are sustained for a sufficient time to permit significant reductions in total IgE levels to occur may be more effective in preventing reinfection than sporadic 'one hit' type anthelmint applications.

An important aspect of our results is that, for the age range that we evaluated, we did not find significant associations between age and either reinfection or total IgE. Also, the differences in the initial total and specific IgE levels between the children who were non-reinfected or subsequently reinfected, were not due to differences in the intensity of infection at the beginning of the study. Thus, at least for the particular conditions of the slum area where the study was undertaken, the results that we obtained were apparently not dependent upon the degree of exposure to infection. In fact, when the children of the study group were considered according to their family units, the associations between high total IgE levels and reinfection became particularly evident. It would be interesting to determine whether genetic factors can influence the propensity of an individual to respond with a polyclonal IgE response to helminth infection, and thus contribute to the over-dispersion of infection intensities that is found in population groups [22].

ACKNOWLEDGMENTS

We thank Mrs Aydee Bolivar for her assistance with the children. This work was supported by CONICIT SI 2030; CDCH 10.35.2072 and Fundación Polar.

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